

09/966, 264 SN
5-2-07

Amendments to the specification: p. 2 line 25

Please replace paragraph [0011] of the specification with the following paragraph:

[0011] FIG. 1: The inversion sequence of the apo-dystrophin-4 cDNA. A cryptic polyadenylation site is underlined at [[+990]] +989.

Please replace paragraph [0025] of the specification with the following paragraph:

[0025] FIG. 6: The full-length apo-dystrophin-4 cDNA and upstream genomic sequence translated. Genomic sequence upstream of the apo-dystrophin-4 cDNA is shown from -233 to -1, the start of the apo-4 sequence. The sequence was subjected to a MacVector alignment and was homologous with the 3' end of the dystrophin cDNA, up until the inversion at 860. A search of the 3' 137 bp of apo-4 alone showed that it was precisely homologous to 3' dystrophin sequence 1.62 Kb downstream in the 3' UTR in the reverse orientation and was thus an inversion of the 3' UTR and genomic sequence. Three potential starting methionines are highlighted at +25, +88, and +100 and a potential CAAT box is underlined at +57. These M's are in phase I of apo-4 alone, but appear in phase II here due to the presence of upstream sequence. The longest open reading frame that should be obtainable from this sequence begins with the M at +88 (33aa). The beginning of exon 79 at [[453]] 451 is also underlined. For a predicted protein in frame 2, a putative transmembrane domain is underlined and putative N-glycosylation sites that follow the Asn-Xxx-Ser/Thr motif are shown (in parentheses when utilized if nonsense suppression occurs). A cryptic polyadenylation site is underlined at [[+990]] +989. The upstream exon identified by GRAIL appears from -90 to [[-5]] -7.

Please replace paragraph [0039] of the specification with the following paragraph:

[0039] FIG. 13: The apo-dystrophin-4 cDNA hybridizes to RNA from apo-4 transfectants. 10 µg of Poly A selected RNA was probed using a full-length apo-dystrophin-4 DNA probe. Lanes from left to right are K562; space; apo-dystrophin COS transfectant; Placenta; and Blondolet (-dystrophin). CDM8 has an endogenous Poly A site 625 bp from the Pst I site at the 3' end of the insert. Transcripts range in size from 1.2-1.62 Kb with a predominant transcript at 1.62 Kb. The apo-4 cDNA contains endogenous Poly A sites at 546 and 632 and the slightly altered sites TATAAA at 849 and 863 bp and AATTAA at [[990]] 989 bp which may account for the smaller signals. The K562 cell line, Placenta and dystrophin-deleted Blondolet cell-line did not hybridize to the probe. The placental RNA was total, as insufficient quantities were available for Poly A selection. The blot was washed twice in 2X SSC, once in 1X SSC and exposed for five hours at -80° C.

Please replace paragraph [0041] of the specification with the following paragraph:

[0041] FIG. 15: Position of RT-PCR primers to analyze potential splice products and to attempt to reproduce the inverted region. Three sets of PCR primers were designed to reproduce the 5' unique 451 bp (F1+R2), the 3' 284 bp including the inversion (F2+R1) and 978 bp of the full-length apo-4 cDNA (F1+R-) (F1+R1) or smaller, potentially spliced products.

Please replace paragraph [0053] of the specification with the following paragraph:

[0053] FIG. 20A. Reduced gel (left to right). Rabbit Reticulocyte Lysate (RRL) (Lanes 1-4)- Hpa I shows no bands; Pst I shows strong signals at 40 Kd (2 Kd smaller than on a non-reduced gel) and 50 Kd (1 Kd) smaller than on a [[Nr]] NR gel) and 25 Kd; the positive control, Bovine Mosaic Virus (BMV) +RRL does not show bands produced in Pst I (RRL) in a less exposed version shown in (FIG. 20B.). Wheat Germ Extracts (WGE) (Lanes 5-8)--only Pst I shows faint broad bands at 34 Kb and 44 Kb, 6 Kb smaller than those in RRL which may reflect differences in glycosylation or an incomplete translation reaction. BMV+WGE shows no band at 33 and a very broad band from 39-46 Kd which is distinct from those in the Pst I lane when compared with the shorter exposure of BMV+WGE in (FIG. 20B.). BMV-WGE shows no bands in the same position as Pst I. Reduced samples had 5% β -ME added before SDS-PAGE. The 10% gel was incubated in Amplify for 30 minutes and exposed at -80° C. for 18 hrs and then for five days at -80° C.

Please replace paragraph [0058] of the specification with the following paragraph:

[0058] FIG 24. This assay shows that purified antibodies do not precipitate the predominant 50 Kd apo-4 transcript as well as the crude antisera, that Fc-CD33 cannot recognize denatured proteins precipitated with P1 and that Fc-CD33 can recognize p50 in COS transfectants.

Please replace paragraph [0059] of the specification with the following paragraph:

[0059] FIG. 25A FIG. 25B: Anti-P1 antisera specifically precipitates a band at p50 in apo-4 transfectants which can be blocked by peptide. NHS-biotin labelled K562 and apo-4 and CD22 transfectants were immunoprecipitated with crude P1 (P1c) or purified (P1p) antisera in the presence and absence of P1 peptide and washed using stringent conditions and ECL detection. p50 was immunoprecipitated with P1c in apo-4 transfectants (Lane 8) and blocked with P 1 peptide (Lane 6) but was not precipitated by CD22 without P1 peptide (Lane 9) or with peptide (Lane 7). P1c also precipitated a band at 50 Kd in K562 (Lane 5) which was partially blocked by peptide (Lane 4), which also appeared in Fc-CD33 immunoprecipitates (Lane 3). P1p precipitated a band at about 58 Kd which was completely blocked by peptide. P1p precipitated a doublet at 62/64 Kd (Lane 2) which was completely blocked by peptide (Lane 1).

Please replace paragraph [0060] of the specification with the following paragraph:

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[0060] ~~FIG. 25B~~ FIG. 25A: Immunoprecipitation of apo-4 under nonreduced and reduced conditions shows possible linkage with a separate subunit. Apo-4 transfectants were labelled with NHS-biotin and immunoprecipitated with the anti-apo 4 P1 antisera (Lanes 1 and 3) and the antisera blocked with peptide (Lanes 2 and 4) and subjected to SDS-PAGE under nonreducing (Lanes 1 and 2) and reducing (Lanes 3 and 4) conditions. Under non-reducing conditions, apo-4 runs at about 62 Kd and a high molecular weight species is blocked at 106-108 Kd (Lane 1). Under reducing conditions, two bands are blocked by peptide at 50 Kd and 56-58 Kd indicating that apo-4 may exist as a heterodimer on the cell surface.

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P. 12 line 27
Please replace paragraph [0066] of the specification with the following paragraph:

[0066] FIG. 27A, 27B and 27C. Five transmembrane domains are predicted for the full-length apo-4S readthrough product. FIG. 27A. The amino acid sequence shows the predicted TM sequence as underlined and the regions against which P1, P2 and P3 peptide antisera were designed in bold and underlined beginning with R at +64 M at +30. Amino acids in which T was changed to A are in bold alone. Structure begins at the "weak" M (+30) included in the P1 peptide antisera followed by the "strong" M at +34.

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P. 15 line 18
Please replace paragraph [0089] of the specification with the following paragraph:

[0089] FIG. 38B. The apo-4 pre-inverted sequence has the basic structure of a retrovirus with the exception of a single direct repeat at the 3' end, which probably accounts for the upstream deletion in apo-4 rather than a complete transposition, and a short 118 bp sequence LTR-like sequence containing some repeats.

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P. 21 line 10
Please replace paragraph [0112] of the specification with the following paragraph:

[0112] Accordingly, the invention relates to a polynucleotide wherein the regulatory element [[that]] regulates the expression of a region of the polynucleotide. Preferably regulation is at the level of transcription or translation, or both.

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P. 74 line 5
Please replace paragraph [0410] of the specification with the following paragraph:

[0410] FIG. 5. Native ICAM-1 precipitates at about 85-110 Kd (Simmons 1988). Fc-CD33Rg precipitated a heavily labelled doublet at 95-100 Kd from the ICAM-1 transfectant (Lane 7). In this assay, ICAM-1 was being used for comparison to the apo-dystrophin-4 cDNA, however, insufficient sample was available for a negative control. Fc-CD33Rg (Lane 5) and Fc-CD33pIg1 (Lane 6) precipitated a band at about 100 Kd from the apo-dystrophin-4 cDNA and fainter bands at average weights of 45, 50 and 66 Kd not seen in the control lane (Lane 4), suggesting that apo-4 could be a low-affinity ligand for CD33. The 66 Kd band is a bit more heavily labelled in Lane 5, suggesting that Fc-CD33 Rg is more efficient at precipitating apo-4. Under stringent washing conditions, Fc-CD33Rg (Lane 2) and Fc-CD33pIg (Lane 3) precipitated

a unique band from K562 at 46 Kd and others, although they appear to be similar to those in the control lane. The bands from Fc-CD33pIg are identical to those precipitated with Fc-CD33Rg except that the 70 Kd band is more heavily labelled, which likely accounts for its slightly higher position on the gel (Lane 3).

P. 75 line 11
Please replace paragraph [0412] of the specification with the following paragraph:

[0412] To compare the apo-dystrophin-4 cDNA to known dystrophin and related cDNAs, the entire cDNA was sequenced in both forward and reverse directions and translated into all three frames (the second one of which is shown in FIG. 6) and the oligonucleotides used to sequence it and to perform PCR appear in the appendix. The cDNA sequence begins at position 1, with additional upstream genomic sequence at -233 to -1 obtained with genomic clones from the 3' intronic region of exon 78, later found to match the region of apo-4. The structure of the 3' approximately 546 bp was compared with the sequencing of genomic phage clone DNA from the 3' region of dystrophin (Kunkel, L. M. et al., Gene, 33: 251-258, 1985) to confirm the precise point of a 137 bp inversion discovered at the 3' end of apo-4. The second reading frame includes both the putative N-terminus of apo-4, an upstream exon identified with GRAIL and the hydrophobic frame of exon 79 spliced onto the 31 3' terminal amino acids of Dp71 (Lederfein 1992) which appear in unspliced apo-4 from 454 bp to 546 bp (FIG. 2) (FIG. 6).

Please replace paragraph [0412] of the specification with the following paragraph:

duplicate
~~[0412] To compare the apo-dystrophin-4 cDNA to known dystrophin and related cDNAs, the entire cDNA was sequenced in both forward and reverse directions and translated into all three frames (the second one of which is shown in FIG. 6) and the oligonucleotides used to sequence it and to perform PCR appear in the appendix. The cDNA sequence begins at position 1, with additional upstream genomic sequence at -233 to -1 obtained with genomic clones from the 3' intronic region of exon 78, later found to match the region of apo-4. The structure of the 3' approximately 546 bp was compared with the sequencing of genomic phage clone DNA from the 3' region of dystrophin (Kunkel, L. M. et al., Gene, 33: 251-258, 1985) to confirm the precise point of a 137 bp inversion discovered at the 3' end of apo-4. The second reading frame includes both the putative N-terminus of apo-4, an upstream exon identified with GRAIL and the hydrophobic frame of exon 79 spliced onto the 31 3' terminal amino acids of Dp71 (Lederfein 1992) which appear in unspliced apo-4 from 454 bp to 546 bp (FIG. 2).~~

P. 91 line 22
Please replace paragraph [0453] of the specification with the following paragraph:

[0453] FIG. 25B. This gel reinforces the finding that p50 is specific to apo-4 transfectants, that it can be blocked by peptide and that a surface-labelled band of the same size

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can be precipitated by P1c and Fc-CD33 in K562, although it is only partially blocked by peptide.

p. 95 line 14
Please replace paragraph [0464] of the specification with the following paragraph:

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[0464] A TopPred product beginning at the first available M at +9 aa, apo-4F (full-length) appears in FIGS. 37A and 37B and also predicts two certain and [[four]] three possible TM domains, although the 31 aa N-terminus is predicted to be cytoplasmic in a putative four-domain structure. An apo-4 product beginning at the M at +34 shares an equivalent predicted structure with apo-4F with an extracellular N-terminus. Whether the N-terminus is extra or intracellular, extracellular recognition could occur via the extracellular portions of the apo-4 protein and/or the N-terminus (FIGS. 37A and 37B). Two other N-glycosylation sites were identified in the full-length apo-4 readthrough product at 93 (NQT), 203 (NKS). In a five TM domain structure, the final two could only be utilized on Apo-4S or F if the N-terminus were intracellular and the first two could only be utilized on Apo-4F if the N-terminus were extracellular. If only the two certain TM domains were utilized and the N-terminus were on the outside, the first two and the last site could be used. N-glycosylation would account for a size discrepancy between an in vitro synthesized protein and one immunoprecipitated from transfectants and cell lines. Peptide sequencing of both major products from transfectants would be the only way to confirm the protein sequence.

line 4 p. 98
Please replace the heading above paragraph [0471] of the specification with the following heading:

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Fc-CD33 ~~Show level~~ Shows Some Level of Apo-4 Staining

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Amendments to the Specification

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5-2-07 Please replace the heading above ~~paragraph 0465~~ of the published application with the following heading:

Apo-4 P1 Antibodies Suggest Specific Staining in ~~Issues~~ Tissues